

## **REMARKS/ARGUMENTS**

Claims 1, 2, 4, 5, 7-12, 42 and 43 are now pending in the application. Claim 6 is canceled without prejudice herein. Claims 1, 7, 9, 10, 42 and 43 are currently amended.

Background literature references noted in the accompanying Declaration under 37 C.F.R. 1.132 by Hal S. Padgett, Ph.D. that were not previously supplied in an Information Disclosure Statement are supplied in the present IDS submission for the convenience of the Examiner.

Support for the amendment to Claims 1, 7 and 9 of “wherein said heteroduplex has at least two mismatch sites separated by at least one complementary nucleotide base pair” is found throughout the specification such as in Figures 1 and 2 as well as in the examples.

Support for the amendment to Claims 1, 7, 9 and 10 of “within six nucleotides 3' of a mismatch site” is found at least in paragraph [00155] “In addition to CEL I, a number of functionally equivalent, and potentially similar activities found in extracts from a variety of plant species (Oleykowski, Nucleic Acids Res 1998; 26:4597-602) may be used. Other mismatch-directed endonucleases such as T4 endonuclease VII, T7 endonuclease I, and SP nuclease (Oleykowski, Biochemistry 1999; 38: 2200-5) may be used. Another particularly useful mismatch-directed endonuclease is RES I”. Support is also found in paragraphs [00176] to [00178] and references cited therein, as well as in paragraph [00179], e.g., “... Endonuclease VII introduces double stranded breaks by creating nicks and counternicks **within six nucleotides 3' of the mispairing ...**”.

Support for the amendment to Claims 42 and 43 of “wherein said activity does not utilize the MutS/L/H enzyme system” is found in paragraph [00180] wherein the instant method is distinguished from methods in which a heteroduplex is exposed to a cellular DNA repair system.

### **Drawings**

Item 3:

Replacement drawings for Figures 1-2 have been submitted, and the corresponding descriptions of the figures in the specification have been amended to describe the corresponding SEQ ID NOs. No new matter was added in these amendments.

It is respectfully requested that this objection be withdrawn.

### **Claim Rejections –35 U.S.C. 112**

Item 4:

Claims 1, 2, 4-6, and 8-12 were rejected under 35 U.S.C. 112, second paragraph, as indefinite over the recitation of the phrase “preparing at least one heteroduplex between said at least two parent polynucleotides, cleaving at least one polynucleotide strand in said heteroduplex at a mismatch site with an effective amount of mismatch-directed strand cleavage activity to form a cleavage site, replacing at least one nucleotide on at least one polynucleotide strand at or near the cleavage site”. The phrase has been amended by the addition of “wherein said heteroduplex has at least two mismatch sites separated by at least one complementary nucleotide base pair” to specify that the heteroduplex must contain at least two mismatch sites.

The Examiner further states “Further the cleaving step would have to be effected on both strands of the heteroduplex...”. Applicants respectfully disagree with this interpretation. There is no reason why the cleaving steps couldn’t occur all on one or the other strand of a particular heteroduplex. If, for example, several mismatch sites on one strand were converted to basepaired sites, while other sites remained mismatched, the resulting strand would not be identical to the parent polynucleotide. Accordingly, withdrawal of this rejection is respectfully requested.

Claim 6 was rejected as indefinite. Claim 6 has been canceled without prejudice, making the rejection moot.

Claim 7 was rejected as indefinite over the phrase “preparing at least two different heteroduplexes among said at least three parent polynucleotides, cleaving at least one polynucleotide strand in said heteroduplexes at a mismatch site with an effective amount of mismatch-directed strand cleavage activity to form a cleavage site, replacing at least one nucleotide on at least one polynucleotide strand at or near the cleavage site”. The phrase has been amended by the addition of “wherein each of said at least two different heteroduplexes has at least two mismatch sites separated by at least one complementary nucleotide base pair”, obviating this rejection.

The Examiner further states “the cleaving step would have to be effected on both strands of the heteroduplex...”. Applicants respectfully disagree with this interpretation. There is no reason why the cleaving steps couldn’t occur all on one or the other strand of a particular heteroduplex. If, for example, several mismatch sites on one strand were converted to basepaired sites, while other sites remained mismatched, the resulting strand would not be identical to the parent polynucleotide. According, it is respectfully requested that this rejection be withdrawn.

Claim 42 was rejected as indefinite over the phrase “mixing copies of the heteroduplex polynucleotide sequence with an effective amount of mismatch-directed strand cleavage activity, proofreading activity, and ligase activity; and allowing sufficient time for a number of non-complementary nucleotide base pairs to be converted to complementary base pairs, wherein a diverse population of polynucleotide sequences results, wherein at least one polynucleotide sequence in said diverse population is not identical to said heteroduplex polynucleotide sequence or the complementary sequence of said heteroduplex polynucleotide”. The claim has been amended to recite that the “at least two non-complementary nucleotide base pairs” are “separated by complementary nucleotide base pairs” making it clear that the heteroduplex has at least two non-complementary sites.

The Examiner further states “the cleaving step would have to be effected on both strands of the heteroduplex...”. Applicants respectfully disagree with this interpretation. There is no reason why the cleaving steps couldn’t occur all on one or the other strand of a particular heteroduplex. If, for example, several mismatch sites on one strand were

converted to basepaired sites, while other sites remained mismatched, the resulting strand would not be identical to the parent polynucleotide.

Additionally, the Examiner notes that the term “mixing” is not clear. The claim has been amended to clarify that the heteroduplex is “exposed” to the agents. In view of the foregoing amendments and arguments, withdrawal of this rejection is respectfully requested.

Claim 43 was rejected as indefinite. The claim has been amended to recite “wherein said heteroduplex has at least two mismatch sites separated by at least one complementary nucleotide base pair” to specify that the heteroduplex must contain at least two mismatch sites.

The Examiner further states “the cleaving step would have to be effected on both strands of the heteroduplex...”. Applicants respectfully disagree with this interpretation. There is no reason why the cleaving steps couldn’t occur all on one or the other strand of a particular heteroduplex. If, for example, several mismatch sites on one strand were converted to basepaired sites, while other sites remained mismatched, the resulting strand would not be identical to the parent polynucleotide.

Additionally, the Examiner notes that the term “mixing” is not clear. The claim has been amended to clarify that the heteroduplex is “exposed” to the agents. In view of the foregoing amendments and arguments, withdrawal of this rejection is respectfully requested.

### **Claim Rejections - 35 U.S.C 102(b)**

Item5:

Claims 1-2, 4, 6-12, and 42-43 were rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Arnold et al. This rejection is respectfully traversed.

Arnold et al. teach a method for evolving a polynucleotide toward acquisition of a desired property by exposing a heteroduplex to a cellular DNA repair system to convert the heteroduplexes to parental polynucleotides or recombined polynucleotide variants. In one method the heteroduplexes are exposed to the host cells' DNA repair system *in vivo*. Arnold

et al. also mention the possibility that a suitable DNA repair system could be prepared in the form of cellular extracts.

Independent Claims 1, 7 and 9 as amended require “cleaving at least one polynucleotide strand in said heteroduplex within six nucleotides 3’ of a mismatch site with an effective amount of mismatch-directed strand cleavage activity to form a cleavage site”. Arnold et al. do not teach such an activity. According to Lahue et al. PNAS 84(1987)1482-1486 (cited in an earlier IDS), the *E. coli* DNA repair system includes a protein that recognizes mismatches but does not cleave (mut S), another protein that cleaves the DNA at a hemimethylated dGATC site, but does not recognize mismatches, and a protein to bridge these two. Other proteins in the *E. coli* repair system described by Lahue et al. include DNA helicase II and single-stranded DNA binding protein (SSB), Exonuclease I for removing large tracts of DNA that may include the mismatch, and the DNA Pol III holoenzyme for resynthesizing the large tracts of DNA. None of these is a mismatch-directed strand cleavage activity that cleaves within six nucleotides 3’ of a mismatch site.

Independent Claims 42 and 43 as amended require “exposing copies of the heteroduplex polynucleotide sequence to an effective amount of mismatch-directed strand cleavage activity wherein said activity does **not** utilize the MutS/L/H enzyme system”. The method of Arnold et al. is thought to require exposing the heteroduplex to the Mut S/L/H enzymes or their homologs to effect cleavage of the heteroduplex (see Arnold et al. page 17, lines 7-12, as well as Volkov, Shao and Arnold, Nuc Acids Res Vol 1999, Vol. 27, No. 18, e18 cited in the instant specification). The instant claims specifically do not use the Mut enzymes.

Further, the specification in Examples 6 and 14 presents negative control reactions in which the heteroduplex is treated with the components of the instant reaction, except for a mismatch endonuclease, prior to the downstream processing step of transformation of the heteroduplex into *E. coli*. (Transformation of heteroduplexes into *E. coli* is taught in references such as Abastado et al., Cami et al. or Chang et al. cited in an earlier IDS and the attached Padgett Declaration.) If the DNA repair system of *E. coli* were to perform the instant process as suggested by the Examiner, then output molecules like those obtained with the instant method would be expected to result, however the data presented in Examples 6 and 14 show that the output molecules of the negative control reaction are not

the same as those of the instant method. Hence, the method of Arnold et al. does not anticipate the instant method.

In addition, the evidence in the Padgett Declaration submitted under 37 C.F.R. 1.132 with this amendment makes it clear that the instant process yields products that are dramatically different from those that are expected simply from treatment of heteroduplexes with a DNA repair system. Indeed, the products from the negative control of the instant method were subsequently transformed into *E. coli* resulting in only low levels of sequence reassortment (an occasional recombined molecule, typically with only one crossover point) which is consistent with what has been reported previously when heteroduplexes with multiple mismatches were exposed to DNA repair systems either *in vivo* (references 1, 2, 3, 4, 9, 10 in the Declaration) or *in vitro* (references 5 and 6 in the Declaration). DNA repair systems have been observed to perform similarly *in vitro* to the way they do *in vivo* (references 5 and 8 in the Declaration). Since a person having ordinary skill in the art would reasonably expect a DNA repair system extract to perform similarly *in vitro* to the way it performs *in vivo*, the system described in the instant claims is dramatically different from both *in vivo* (living cells) and *in vitro* (cell extract) DNA repair systems.

For these reasons at least, Arnold et al. do not anticipate the instant claims. Reconsideration and withdrawal of the rejection is respectfully requested.

#### **Claim Rejections - 35 U.S.C 103(a)**

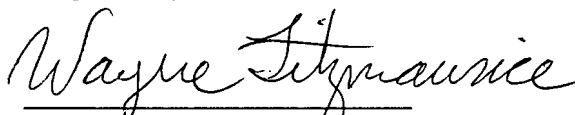
Item 6:

Claim 5 was rejected under 35 U.S.C. 103(a) over Arnold et al. in view of Wetmur. This rejection is respectfully traversed. As detailed above, the primary reference of Arnold et al. fails to teach a method of preparing a variant polynucleotide that has a nucleotide sequence not identical to either of said at least two parent polynucleotides as defined in the instant claims as amended. The secondary reference of Wetmur does not compensate for this basic deficiency of Arnold et al., hence the teaching in Wetmur of a heteroduplex comprising an entire genome is moot. According, it is respectfully requested that this rejection be withdrawn.

Claims 1, 2, 4, 5, 7-12, 42 and 43 are now pending in the application. In view of the Padgett Declaration under 37 C.F.R. 1.132, current claim amendments and comments above, the rejections have been overcome. Reconsideration, withdrawal of the rejections and early indication of allowance are respectfully requested. If any issues remain, the examiner is encouraged to call the undersigned for prompt resolution.

If needed, Applicants petition for an extension of time under the provisions of 37 CFR 1.136(a) for sufficient time to accept this response. The commissioner hereby is authorized to charge payment of any fees under 37 CFR § 1.17, which may become due in connection with the instant application or credit any overpayment to Deposit Account No.500933.

Respectfully submitted,



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Enclosures: Petition for a Two-Month Extension of Time (1 page)  
Replacement Sequence Listing (38 pages)  
CRF Sequence Listing on CD (2 copies)  
Statement that CRF and paper copy are the same (1 page)  
Replacement drawings for Fig. 1 and Fig. 2 (2 sheets)  
Declaration by Hal S. Padgett, Ph.D. (14 pages)  
Figures supporting Declaration (5 sheets)  
Information Disclosure Statement supporting Declaration (2 pages)  
Copies of References in IDS  
Self-addressed postcard

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